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16. ABSTRACT

Liquid from canine airway surfaces was absorbed onto filter paper strips and analyzed. In resting conditions, tracheal surface liquid was hyperosmolal (330 mosmol/kg H_2O) compared to plasma with raised Na^+ (158 meq/l), Cl^- (134 meq/l), K^+ (28 meq/l), and HCO_3^- (32 meq/l) concentrations. The volume collected was increased by repetitive sampling, a response blocked by atropine, or by methacholine injection. Compared to nose breathing, tracheal surface liquid osmolality was increased by 10 min of mouth breathing (410 mosmol/kg H_2O). Surface liquid from 0.5-cm diameter bronchi was nearly isosmolal (304 mosmol/kg H_2O) with plasma in resting conditions, with Na and Cl concentrations lower that plasma (120 and 106 meq/l, respectively), and K^+ (52 meq/l), and HCO_3^- (50 meq/l) concentrations higher that those of plasma or tracheal liquid. Although the K^+ in tracheal fluid approaches the value for electrochemical equilibrium, K^+ in fluid from the bronchi and HCO_3^- in both regions cannot be accounted for by passive forces. The regional difference in osmolality supports the concept that the higher osmolality of tracheal liquid reflects evaporative water loss from this site. The transepithelial osmotic gradient generated by evaporative water loss may be a driving force for hydration of the tracheal surface.

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Regional differences in airway surface liquid composition

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BOUCHER, R. C., M. J. STUTTS, P. A. BROMBERG, AND J. T. GATZY. *Regional differences in airway surface liquid composition*. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 50(3): 613–620, 1981.—Liquid from canine airway surfaces was absorbed onto filter paper strips and analyzed. In resting conditions, tracheal surface liquid was hyperosmolal (330 mosmol/kg H_2O) compared to plasma with raised Na^+ (158 meq/l), Cl^- (134 meq/l), K^+ (28 meq/l), and HCO_3^- (32 meq/l) concentrations. The volume collected was increased by repetitive sampling, a response blocked by atropine, or by methacholine injection. Compared to nose breathing, tracheal surface liquid osmolality was increased by 10 min of mouth breathing (410 mosmol/kg H_2O). Surface liquid from 0.5-cm diameter bronchi was nearly isosmolal (304 mosmol/kg H_2O) with plasma in resting conditions, with Na and Cl concentrations lower than plasma (120 and 106 meq/l, respectively), and K^+ (52 meq/l), and HCO_3^- (50 meq/l) concentrations higher than those of plasma or tracheal liquid. Although the K^+ in tracheal fluid approaches the value for electrochemical equilibrium, K^+ in fluid from the bronchi and HCO_3^- in both regions cannot be accounted for by passive forces. The regional difference in osmolality supports the concept that the higher osmolality of tracheal liquid reflects evaporative water loss from this site. The transepithelial osmotic gradient generated by evaporative water loss may be a driving force for hydration of the tracheal surface.

trachea; bronchi; osmolality; electrolytes

RECENT REPORTS SUGGEST that active Na^+ and Cl^- transport by the respiratory epithelium participates in regulating the volume and composition of the surface liquid that lines the conducting airways (2, 25). In vitro studies of short-circuited canine trachea have demonstrated an active translocation of Cl^- toward the lumen and a smaller flow of Na^+ toward the submucosa, directions of transport that may contribute to the potential difference (PD) of about −30 mV under open-circuit conditions (25). However, because the airways are not normally fluid filled and their architecture is complex, it is difficult to design experiments to directly assess ion movements in vivo. The PD recorded in vivo in dogs is similar to that measured in vitro, suggesting that comparable active ion translocation may occur in intact animals (6). Another indirect approach to the assessment of the role of the epithelium in the regulation of airway surface liquid is to examine its composition. This composition, in conjunction with the transmural electric PD, may provide clues to transport mechanisms in vivo. However, analyses of airway liquid composition have been hampered by the thin fluid layer and the small volume of liquid available for sampling. Adams et al. (1) approached this problem by positioning a cylindrical screen in the trachea of dogs and collecting airway liquid at 1-h intervals. We recovered liquid from filter paper strips that were applied locally to the surface of the large airways. This is a simple rapid technique, which has been previously employed for sampling nasal secretions (19), and yields values of tracheal surface liquid Na^+, Cl^+, and osmolality that agree well with those from fluid retained on the implanted screen (21). In addition, we have extended the use of this technique to measure surface liquid K^+, HCO_3^-, and Ca^{2+} and to characterize regional differences in the composition of liquid from the surface of the trachea and ~5-mm diameter bronchi.

METHODS

Protocol. Male mongrel dogs, 11–22 kg, were used in most studies. In addition, three female beagles (10 kg), housed in isolation from birth and subsequently shown to have no detectable tracheal infection (6), were studied. The animals were anesthetized with intravenous pentobarbital sodium (25 mg/kg) and a shortened endotracheal tube was inserted. Pieces of ashless filter paper (Whatman 541, Whatman, England) were tared in capped, acid-washed glass flasks and then placed on the airway surface with biopsy forceps immediately after intubation. Each filter paper remained in contact with the airway surface for 1 min, was withdrawn, and was immediately reweighed in the capped glass flask. Our initial studies were designed to compare the composition of tracheal liquid with that of saliva and plasma. Samples of sublingual saliva were absorbed into three filter-paper pledgets (1 × 4 cm, 40 mg). Another three pledgets of the same size were placed sequentially on the tracheal surface. Placement of each pledget was viewed through a bronchoscope to ensure sampling at separate tracheal sites. Collection of the three samples required less than 5 min. Fifty-microliter samples of freshly drawn plasma were added to filter paper or added directly to acid-washed flasks. In some experiments both tracheal and bronchial liquids were sampled. Biopsy forceps were positioned in both

regions under fluoroscopic visualization. To avoid admixture of tracheal with bronchial liquid, the bronchial pledget (0.2 × 4 cm, 8 mg) attached to the forceps was contained in a polyethylene catheter (OD, 0.5 cm) with the distal orifice occluded with Parafilm. After the trachea and mainstem bronchi were cleared, the Parafilm cap was dislodged and polyethylene catheter was advanced until slight resistance was noted. Then the filter-paper pledget was advanced 3 cm beyond the orifice. Because of the small quantities of bronchial liquid available, six bronchial samples, two contemporaneous with each tracheal sample, were obtained for analysis.

Two series of experiments evaluated the effects of interventions on tracheobronchial liquid. In one series, after the control samples were obtained, the animal was extubated and either nose or mouth breathing was instituted, or methacholine (0.2 mg/kg sc) or atropine (1.0 mg/kg iv) was injected. Ten minutes later the dogs were reintubated and serial samples were obtained from different airway sites than the initial series of samples. Because serial sampling appeared to induce changes in the volume and composition of tracheobronchial liquid (see RESULTS), a second series of experiments was performed in which the control sampling period was omitted and samples were obtained 10 min after institution of mouth breathing or injection of methacholine. The choice of drug doses and the sampling interval was guided by the reports of others (29).

Six filter-paper samples from separate dogs were examined by scanning electron microscopy (SEM). Each pledget was fixed with aerosolized formaldehyde/paraformaldehyde immediately after withdrawal, postfixed in this fixative, and processed (24). ^{51}Cr-labeled red cells that were directly applied to filter paper, fixed, and processed in an identical manner served as a control for cell loss during fixation and processing.

The ranges of laboratory humidity and temperature were 40–65% and 23–26°C, respectively.

Analyses. Repetitive weighing of flasks that contained filter paper agreed within 0.5 mg. Addition of liquid to the filter paper did not alter reproducibility. Filter-paper pledgets were dipped in saline and weighed. The pledgets used to sample tracheal liquid were able to absorb around 200 mg, whereas the smaller bronchial pledget absorbed about 50 mg. Sample volume was defined as the difference between wet weight and tared weight.

We tried to obtain at least three liquid samples from each airway region during each sampling period. The order of subsequent sample analysis was independent of sampling sequence. The sample in one flask was extracted into 0.8 ml of double-distilled water by vortex mixing over a 2-h period. Total protein in a 0.1-ml aliquot was determined by the technique of Lowry et al. (20). Dog albumin was used as a standard. Another aliquot (0.025 ml) was reserved for albumin determination by a solid-phase radioimmunoassay employing a rabbit anti-dog albumin antiserum. A 0.5-ml aliquot was removed for measurement of osmolality by freezing-point depression (model 2007, Precision Systems, Sudbury, MA). Periods of water extraction were limited to 2 h because longer extractions were accompanied by a progressive increase in osmolality that was not accounted for by evaporative loss. It is likely that the increase resulted from proteolysis (30). The osmolality of canine plasma (50 μl) that was applied to filter paper and subsequently extracted for this interval was not different from that of plasma, which was measured directly. Replicate measurements of freezing point agreed within 1 mosmol/kg H_2O so that the reproducibility of a 50-μl sample is ±17 mosmol/kg H_2O. The filter paper in the second flask was shaken with 5 ml of 0.1 N Ultrex HNO_3 (Baker Chemicals) for 48 h. The extract was transferred to a 10-ml volumetric flask and diluted to volume. A filter-paper blank and papers with standard solutions were carried through the extraction procedure. Na^+ and K^+ concentrations of the diluted extract were determined by flame photometry without internal standard (Coleman model 21, Perkin-Elmer, Brook, IL). Ca^{2+} was initially measured by a chemical technique (4). However, these measurements were not sensitive enough to assess the Ca^{2+} in small sample volumes. Most Ca^{2+} concentrations were determined by flameless atomic absorption spectrometry (model 305B with HGA-2100 Graphite Furnace Controller, Perkin-Elmer, Norwalk, CA) at 422.7 nm. Chloride was measured by amperometric titration of an aliquot of the HNO_3 extract (Chloridometer, Buchler, Fort Lee, NJ). HCO_3^- in the contents of the third flask was measured by a modification of the method of van Slyke and co-workers [see Bitner and Hall (5)]. A standardized HCl solution with a screened indicator was shaken with the samples, filter paper with known amounts of added HCO_3^-, or filter paper blanks and then back-titrated with NaOH (Oxford Titrator, Oxford Laboratories, Foster City, CA). When the volume of liquid was less than 15 mg, the coefficient of variation of HCO_3^- concentrations of standard solutions was unacceptable (>±15%). Accordingly, the HCO_3^- concentrations of biologic fluid samples smaller than 15 mg are not reported.

Paired *t* tests were used to estimate the statistical significance of differences between the composition of liquids from the same airway region of the same animal that were sampled at different times. Differences among the composition of airway liquid, saliva, and plasma were compared by both unpaired and, where appropriate, paired *t* tests.

RESULTS

A summary of the electrolyte and protein concentrations and volume of tracheal liquid, saliva, and plasma is shown in Table 1. Volumes noted in Table 1 and in Tables 2–6 (except line two of Table 6) represent the sum of liquid recovered on three (trachea) or six (bronchi) filter papers. Plasma concentrations of electrolytes and total protein and plasma osmolality are similar to published values (3). Further, plasma HCO_3^- from the titration and that calculated from arterial blood gas analyses were comparable (mean arterial pH = 7.40, P_{CO_2} = 43, n = 13). Plasma albumin values measured by radioimmunoassay were indistinguishable from values obtained by high voltage cellulose acetate electrophoresis (2.8 vs. 2.7 g/100 ml, respectively). The measured composition of saliva lies within the range of values reported for unstimulated canine salivary secretions (31, 33). The values for

TABLE 1. *Composition of canine body liquids*

Source	Site	n	Osmolality, mosmol/kg H_2O	Σion	meq/l				mmol/l	Total Protein, g/100 ml	Albumin, g/100 ml	Vol, mg
					Na^+	K^+	Cl^-	HCO_3^-	Ca^{2+}			
Mongrel	Trachea	47	330.1*†	358	158.4*†	28.9†	134.4*†	36.6*†	6.85†‡	2.70*†	0.37*†	131.4
			±14.9		±7.2	±2.0	±7.5	±2.9	±1.01	±0.23	±0.06	±16.2
Beagle	Trachea	3	344.6		173.0	49.7	156.0			2.31		150.5
			±17.6		±28.7	±9.4	±30.0			±0.80		±62.8
Mongrel	Saliva	31	261.0†	238	86.3†	30.5†	83.3†	32.4†	5.71	0.52†	0.01 b	129.1
			±17.4		±4.9	±1.7	±5.8	±2.6	±2.50	±0.06	±0.004	±10.3
Mongrel	Plasma	33	296.2†	289	141.6	5.2	111.7	26.4	4.16	6.94	2.8	
			±4.3		±3.0	±0.2	±2.4	±0.9	±1.8	±0.15	±0.8	

Values are mean ± SE; n, no. of observations. * Significantly different ($P < 0.05$) from saliva. † Significantly different ($P < 0.05$) from plasma. ‡ $n = 18$.

TABLE 2. *Composition of simultaneously sampled tracheal and bronchial liquid*

Source	Site	n	Osmolality, mosmol/kg H_2O	Σion	meq/l				mmol/l	Total Protein, g/100 ml	Albumin, g/100 ml	Vol, mg
					Na^+	K^+	Cl^-	HCO_3^-	Ca^{2+}			
Mongrel	Trachea	18	345.1 ± 14.1	353	145.4 ± 14.4	26.8 ± 3.1	142.4 ± 13.7	31.5 ± 5.9	6.85 ± 1.01	3.92 ± 0.33	0.49 ± 0.07	157.3 ± 28.4
Mongrel	Bronchi	18	304.2 ± 4.6	321	120.8 ± 12.8	36.8 ± 4.5	105.7 ± 4.7	52.2 ± 10.4	6.53 ± 1.19	4.80 ± 0.25	0.55 ± 0.09	80.0 ± 3.6
	P*		<0.01		<0.01	<0.01	<0.01	<0.01	NS	NS	NS	

Values are mean ± SE. NS, not significant. * t test for paired comparisons.

Na^+, Cl^-, HCO_3^-, and protein concentrations, and osmolality, indicate that tracheal liquid can be distinguished from saliva. Compared to plasma, tracheal liquid is significantly hyperosmolal. Na^+, K^+, HCO_3^-, Cl^-, and Ca^{2+} concentrations are higher whereas albumin concentrations, as previously reported (26), are lower than those of plasma.

The composition of simultaneously sampled tracheal and bronchial liquid and P values for paired comparisons are shown in Table 2. Bronchial liquid was characterized by lower Na^+ and Cl^- and higher K^+ and HCO_3^- concentrations than tracheal liquid or plasma, resulting in a liquid that was nearly isosmolal with plasma. No differences were detected between Ca^{2+} or albumin concentrations in tracheal and bronchial liquids.

The higher tracheal and bronchial K^+ concentrations measured by this technique could reflect the K^+ content of mucosal cells and/or cells that enter the airway lumen in response to infections of the respiratory tract. Tracheal liquid composition was measured in disease-free beagle dogs (Table 1). All values, and in particular the K^+ concentration, were similar to those from our mongrel population that characteristically exhibits a high incidence of infection (18). In addition, filter paper pledgets were fixed for SEM and examined. Few cells were found in pledgets from six dogs (Fig. 1). The appearance of the material adherent to the filter paper after fixation resembles the "mucus blanket" observed by Sturgess (35) in scanning electron micrographs of rabbit airway surfaces. The relative absence of cells is not an artifact of preparative procedure because more than 75% of the ^{51}Cr-labeled red cells that were applied to the filter paper adhered to the pledgets at the end of processing ($n = 3$).

When surface liquid sampling was separated by a 10-min interval, the second samples from both airway regions (Table 3) were characterized by a higher total volume and K^+ and HCO_3^- dilution, without changes in osmolality. It was possible that the sampling process

FIG. 1. Scanning electron micrograph of filter paper after removal from airway surface. (×8,400.)

irritated afferent receptors so that vagal reflex mechanisms may have contributed to the alterations in liquid composition and volume. Because the efferent limb of such a reflex may release a cholinergic transmitter, we tested the effects of a cholinergic antagonist. The results with atropine (1 mg/kg iv) are shown in Table 4. The increase in sample volume and K^+ and HCO_3^- dilution were blocked by atropine in the trachea but not in bronchi. The volume of liquid collected from methacholine-treated animals was greater than the control period volumes (Table 4), and filter papers were often completely saturated. Because liquid volumes from the second sampling from untreated animals (Table 3) were also higher, these findings were difficult to interpret. How-

ever, methacholine increased the volume and appeared to dilute K^+, HCO_3^-, total protein, and albumin concentrations as compared to controls, when tracheal liquid was sampled during a single perioid 10 min after methacholine administration (see below).

Changes induced by the stimulus of the first sampling subjects a sequential assessment of the effects of mouth breathing on airway liquid composition to the uncertainties noted for drug treatment. Mouth breathing had been shown by Man et al. (21) to increase the osmolality of liquid that accumulated in a cylindrical screen placed on the surface of the canine tracheal mucosa in vivo. We examined the effects of breathing ambient room air by mouth on the composition of saliva and tracheal liquid (Table 5). Mouth breathing resulted in a significant increase in the electrolyte concentrations of saliva and a trend towards raised volumes, osmolalities, and protein concentrations of tracheal liquid. By comparison, the osmolalities of tracheal and bronchial liquid after nose breathing for 10 min were not raised (Table 3). The increase in volume associated with the second sample after mouth breathing (Table 5) suggested that the increase in the osmolality of surface liquid may have been blunted by this effect. The results of single sample experiments are shown in Table 6. Osmolality in these experiments is 410 mosmol/kg H_2O, a value significantly higher than those obtained by repetitive sampling.

TABLE 3. *Changes in airway surface liquid composition induced by 10 min of nasal breathing*

n	Site	Osmolality, mosmol/kg H_2O	meq/l				Total Protein, g/100 ml	Albumin, g/100 ml	Vol, mg
			Na^+	K^+	Cl^-	HCO_3^-			
8	Trachea	−4.8	−4.3	−8.8	+0.1	−20.2	−0.23	+0.12	+63.1
		±17.3	±11.5	±14.4	±9.9	±7.0	±0.40	±0.10	±17.0
	P*	NS	NS	<0.05	NS	<0.05	NS	NS	<0.01
8	Bronchi	−10.8	+13.4	−10.5	+3.7	−17.6	−0.74	+0.11	+45.9
		±14.7	±45.2	±5.8	±10.6	±6.5	±0.48	±0.06	±11.5
	P*	NS	NS	NS	NS	<0.05	NS	NS	<0.01

Values are mean change ± SE. NS, not significant. * Paired comparisons of the difference between samples taken before and 10 min after nasal breathing.

TABLE 5. *Changes in surface liquid composition induced by 10 min of mouth breathing*

n	Site	Osmolality, mosmol/kg H_2O	meq/l				Total Protein, g/100 ml	Vol, mg
			Na^+	K^+	Cl^-	HCO_3^-		
7	Trachea	+49.7	+8.9	−5.4	+3.1	−3.6	+0.52	+36.6
		±73.0	±31.0	±2.7	±35.3	±11.4	±0.21	±39.2
	P*	NS†	NS	0.08	NS	NS	<0.01	0.08
7	Saliva	+191.6	+45.3	+9.6	+9.0	+15.9	+0.25	−11.7
		±55.4	±15.6	±7.7	±22.9	±13.3	±0.11	±22.8
	P*	<0.02	<0.05	NS	NS	NS	<0.05	0.06

Values are mean change ± SE. * Paired comparisons of samples taken before and after 10 min of mouth breathing. † $P > 0.01$.

TABLE 4. *Changes in airway surface liquid composition induced by drug administration*

Drug	Site	n	Osmolality, mosmol/kg H_2O	meq/l					Total Protein, g/100 ml	Albumin, g/100 ml	Vol, mg
				Na^+	K^+	Cl^-	HCO_3^-	Ca^{++}			
Atropine, (1.0 mg/kg iv)	Trachea	9	+20.8	+31.9	−8.2	+13.6	+11.2	−4.3	−0.88	−0.12	−22.3
			±36.4	±17.1	±4.3	±13.8	±5.2	±4.0	±0.26	±0.14	±12.1
	P*		NS	NS	0.08	NS	NS	NS	<0.01	NS	NS
	Bronchi	4	+70.5	−5.0	−11.5	+6.8	−18.8	+0.4	−0.81	+0.15	+52.1
			±41.9	±13.7	±4.7	±9.9	±3.2	±0.9	±0.15	±0.12	±18.9
	P*		NS	NS	0.09	NS	<0.02	NS	NS	NS	<0.07
	Saliva	7	+69.9	+97.9	+17.6	+60.0	+5.5		+0.58	+473	−51.9
			22.9	31.6	8.6	±26.3	±2.0		±0.21	±228	±11.5
	P*		<0.02	<0.02	0.08	0.04	NS	NS	<0.05	<0.05	<0.01
Methacholine, (0.2 mg/kg SC)	Trachea	11	−15.1	−52.9	−15.5	−13.6	−12.5	−4.0	−1.37	−0.24	+239.7
			±40.8	±20.4	±3.4	±19.1	±5.8	±1.7	±0.53	±0.10	±55.8
	P*		NS	<0.05	<0.01	NS	<0.05	0.06	<0.05	<0.05	<0.01
	Bronchi	6	+14.3	+1.6	−36.5	+18.8	−28.1	−0.6	−1.33	+0.15	+99.7
			±11.8	±41.1	±6.0	±5.9	±13.7	±4.0	±0.94	0.27	44.4
	P*		NS	<0.01	<0.01	<0.05	NS	NS	NS	NS	<0.02
	Saliva	4	−69.6	−14.2	−18.4	−6.3	0		−0.41		+199.8
			±61.4	±15.1	±4.9	±13.8	±2.8		±0.15		±44.6
	P*		NS	NS	<0.05	NS	NS		<0.05		<0.01

Values are mean change ± SE. NS, not significant. * Paired comparisons of the difference between samples taken before and 10 min after drug administration. † NS, $P > 0.01$.

DISCUSSION

The physical state of the liquid layer lining the conducting airways remains undefined (16, 36). Accordingly, surface liquid collection techniques cannot be expected to precisely identify the source of the sampled liquid. From the appearance of the filter-paper sample (Fig. 1), it is likely that at least a portion of the sample that adheres to the paper contained mucus glycoproteins. The similarity between the NaCl composition and osmolality of tracheal liquid obtained by our technique and values of Man and co-workers (21) suggests that we sampled a liquid similar to their operationally defined "periciliary liquid." Further, because virtually all NaCl in mucus pellets can be extracted by aqueous wash (21), binding of univalent electrolytes cannot be tight so that any differences between ion concentrations of sol (hypophase) and

TABLE 6. *Composition of tracheal surface liquid after methacholine or 10 min of mouth breathing*

	n	Osmolality, mosmol/kg H_2O	meq/l				Total Protein, g/100 ml	Albumin, g/100 ml	Vol, mg
			Na^+	K^+	Cl^-	HCO_3^-			
Methacholine	6	322.6 ± 8.2*	150.1 ± 16.8	20.9 ± 2.6	132.6 ± 7.0	22.9 ± 3.9*	1.88 ± 0.52	0.17 ± 0.08	245.8 ± 67.8*†
Mouth breathing	7	410.0 ± 17.0*					3.26 ± 0.36	0.62 ± 0.18	20.6 ± 2.2*‡

Values are mean ± SE. * $P < 0.05$, compared by unpaired t tests with the mean from Table 1. † Three filter papers. ‡ Single filter paper.

gel (mucus) would be expected to reflect a Donnan equilibrium.

The composition and volume of airway liquid may have been affected by several factors that are inherent in the protocol and analyses. First, liquid was sampled from anesthetized intubated animals. Pentobarbital sodium, the most frequently used anesthetic in this study, has been reported to exert an atropinelike effect (14). However, Adams et al. (1) found that, compared to halothane anesthesia, pentobarbital did not decrease the collection rates of airway liquid in dogs. Further, we found in preliminary studies that, although chloralose and urethan anesthesia induced an increased flow of saliva, the volume and composition of tracheal liquid was not different from those from pentobarbital anesthetized animals. Finally, atropine (Table 4) reduced the collected volume of saliva and tracheal liquid so that any atropinelike actions of pentobarbital could not have been maximal. Accordingly, it seems unlikely that compared to other anesthetic agents, pentobarbital substantially alters surface liquid volume or composition.

The HCO_3^- analysis was volume limited; i.e., unacceptable variances were noted below 15-μl sample volume. If small sample volumes most closely represent the normal condition, then our sample selection biases the results from regions with small resident volumes such as the bronchi. Unfortunately, few previous values of airway liquid HCO_3^- are available for comparison. Even though it is difficult to estimate the regional P_{CO_2} in airway liquid, Pennington and Reynolds (26) have reported canine airway liquid pH values of 7.8–8.0, a range that is compatible with a HCO_3^--rich liquid.

Similarly, our initial Ca^{2+} measurements by chemical techniques were biased by small volumes and the concentration appears to have been overestimated (10). Subsequent analyses of samples by graphite furnace atomic absorption spectroscopy revealed airway liquid Ca^{2+} concentrations 4–6 meq/l higher than that of plasma. Reports of Ca^{2+} content in canine airway liquid are not available, but several explanations may be offered for the slightly higher Ca^{2+} values. Jayaram and Al-Bazzaz (15) have reported a small but detectable active translocation of Ca^{2+} into the lumen in short-circuited canine trachea preparations mounted in Ussing chambers. In addition, binding to constituents of surface liquid could contribute to differences in total Ca^{2+} concentration. We did not assess free and bound fractions of Ca^{2+}. Whereas low tracheal liquid albumin, compared to plasma, might imply an increased fraction of ionized Ca^{2+}, Ca^{2+} binding to mucus glycoproteins would tend to lower ionized Ca^{2+}.

The high K^+ concentrations in airway liquids reported in Tables 1 and 2 are not unique. Others have noted K^+ concentrations in human and canine secretions that exceed the plasma K^+. For example, the K^+ concentration in secretions from tracheostomized patients averaged ~15 meq/l (27). The K^+ concentration in canine bronchial liquid obtained by Connolly et al. (11) by a technique similar to ours averaged 14 meq/l. Indeed, the low Na-to-Cl ratio in tracheal liquid compared to plasma that was reported previously (21) may reflect the presence of unmeasured K^+ in this liquid rather than, as the authors suggest, evidence for active Cl^- secretion.

The genesis of the relatively high K^+ concentration of both tracheal and bronchial liquid may represent the contribution of several processes. First, it is possible that the K^+ content of cells that adhere to filter paper is extracted by HNO_3^-. These cells might be inflammatory or respiratory in nature. The observation that K^+ concentrations were at least as high in tracheal liquid from uninfected beagles argues against a significant contribution from inflammatory cells. Scanning electronmicroscopy of filter-paper samples showed only rare adherent cells, and these did not appear to be of epithelial origin. Further, the pattern of ion concentrations in airway surface liquid is that of a noncellular liquid (Tables 1 and 2). Cell contents are generally bicarbonate-poor in contrast to the fluid we sampled. Furthermore, the sum of the measured cations closely approximates the measured sum of the anions, leaving a relatively small anion gap. Because cellular ion balance is generally characterized by a significant anion gap, i.e., intracellular cations are balanced by polyanionic macromolecules, the absence of a gap suggests that the contribution of the contents of adherent cells to the electrolyte composition of airway liquid was small. However, we cannot exclude damage to surface cells that releases cell K^+ in exchange for extracellular Na^+.

It is more likely that the high K^+ concentration reflects passive forces and/or active secretion. The transmural electric PD and binding to mucus glycoproteins are possible passive factors that favor an asymmetric transepithelial K^+ distribution. As noted above, significant monovalent cation binding to mucus glycoproteins seems unlikely. In the trachea, the transepithelial PD of 30 mV (lumen negative) would support, at most, a threefold higher K activity with respect to plasma (interstitial fluid) at equilibrium. Because active translocation of K^+ cannot be measured in the excised canine trachea in vitro (8), the PD may account for a portion of the K^+ concentration in tracheal liquid.

On the other hand, passive distribution of K^+ across the airway epithelium cannot explain the K^+ concentration of canine bronchial liquid. The PD in these structures (6–8 mV, lumen negative) is significantly lower than the transtracheal PD (6). Were K^+ to be passively distributed, the PD would support a K^+ gradient between

lumen liquid and plasma interstitium of only 1.4. Moreover, there is recent evidence for active K^+ secretion in canine bronchi (8) that may provide a source for the high K^+ concentration of bronchial liquid and, as surface liquid moves toward the mouth, of tracheal liquid as well.

Whereas systematic errors in the analyses of liquid composition were probably small, the volume of sample trapped by the filter paper must be considered to reflect, at best, a semiquantitative index of the volume of resident airway liquid. In particular, the technique will underestimate the volume when large amounts of liquid are present on the surface. For example, after methacholine challenge some filter papers were completely saturated during the 1-min sampling period.

The total volume of the second liquid samples from the airways was consistently larger than the initial sample (Table 3). Adams et al. (1), also showed that the rate of volume collection was related to the duration of implantation of the collection screen. We found the increase in volume to be associated with lower K^+, HCO_3^-, and protein concentrations. These findings can be explained by the addition of isotonic NaCl solution to the airway surface. It was noted that coughing was induced occasionally during tracheal sampling and induced regularly when carinas were passed during bronchial sampling. These observations raised the possibility that vagal reflex mechanisms may have induced mucosal secretion of an isotonic salt solution. Acetylcholine has been shown in vitro to stimulate net NaCl secretion toward the lumen in excised canine tracheal preparations, an effect that is blocked by atropine (22). Atropine blocked the increase in the volume of the second sample of tracheal liquid and reduced both the volume of collected saliva and the expected reductions in K^+, HCO_3^-, and total protein concentrations noted in Table 3. No data are available to indicate whether this cholinergic-mediated secretion is derived from glandular or surface epithelial cells, or both.

A leak of interstitial fluid into the lumen is another possible source of the increased volume of the samples collected after the initial sampling period. Although the albumin concentration did not increase significantly in the second samples (Table 3), the increase in collected volume suggests an increase in total albumin flow toward the airway surface. This response could result from local trauma and/or from the initiation of a reflex. Even a light touch with a cotton swab is sufficient to disrupt epithelial tight junctions of the rat trachea (23). These junctions are thought to be the barriers that limit passive albumin movement from the interstitium into the lumen. Therefore, even minor epithelial trauma induced by placement of the filter-paper pledgets may have produced local changes in luminal liquid. Reasor et al. (29) have shown that methacholine administration in dogs induces albumin flow into the tracheal lumen. The volume and albumin concentrations of the fluids collected after methacholine (Table 4) and the inhibition of the resampling effect in the trachea by atropine are compatible with this interpretation. There are no data that rule out the participation of cholinergic mediators in structural changes induced by local irritation.

In four experiments where bronchial liquid was measured after atropine administration, the volume increase and dilution of K^+, HCO_3^-, and protein response was not blocked. We have noted in in vitro studies with bronchi that, compared to trachea, addition of acetylcholine to the submucosal bathing solution reduces NaCl reabsorption but does not induce net secretion into the lumen (9). These results suggest that most of the increase in the volume of bronchial liquid induced by sampling may be mediated by local trauma, noncholinergic transmitter(s), or neurohumor(s).

A sampling procedure that initiates alterations in airway liquid severely limits the evaluation of the effects of other acute perturbations when sequential samples must be compared with an initial control period. For example, since methacholine was administered after an initial sample was taken, it is difficult to separate the effects of irritation, i.e., sampling, from those of methacholine. When the sampling period before methacholine administration was omitted, the volume collected after drug injection was much larger than expected (compare line 1, Table 1 with line 1, Table 6). These results indicate that the direction of the changes induced by irritation and cholinergic stimulation are similar.

Our study supports the concept that the trachea may serve to complete the humidification of inspired air and offers a mechanism for water addition to the tracheal surface. The osmotic gradient between trachea and plasma probably reflects the evaporative loss of water from the tracheal surface into inspired air. Man and coworkers (21) have shown that tracheal surface liquid osmolality can be nearly isosmolal to plasma when the relative humidity of inspired air at body temperature is nearly 100% and that exposing the tracheal surface to "drier" inspired air, i.e., via mouth breathing, increased surface liquid osmolality to 380 mosmol/kg H_2O, a finding confirmed in the present study (Tables 5 and 6). Further, there is no evidence to suggest that tracheal epithelia can elaborate a hypertonic solution.

The presence of an osmotic gradient may provide a driving force for water movement onto the tracheal surface. A reflection coefficient of 0.08 for NaCl can be calculated from the tracheal equivalent pore radius (7) and the relationship that describes the movement of solute and solvent through pores (34). This value along with a transepithelial 40 mosmol/kg H_2O gradient would generate a 70 cmH_2O osmotic driving force for flow toward the lumen. If the combination of negative interstitial pressure (17) and the colloid gradient between interstitium and lumen are assumed to approximate 20 cmH_2O, then the net driving force (50 cmH_2O) coupled with a range of hydraulic conductivity (32) that is representative of gall bladder, choroid plexus, intestine, and proximal and distal nephron, and the tracheal surface area (~60 cm^2) can account for the addition of 0.1–1.5 ml of water to the inspired air per hour. A preliminary estimate of the pressure filtration coefficient (L_p) for trachea (39) falls at the lower end of the range mentioned above, but this value was calculated from osmotic flow induced by sucrose. The reflection coefficient for sucrose can be estimated from the equivalent pore radius of the tracheal barrier to be 0.11 rather than the value of 1.0 assumed by Welsh and co-workers (39). Consequently, L_p for the trachea may have been underestimated. Active

NaCl secretion may contribute little to water movement at rest because Cl^- secretion has not been detected in vitro under open circuit, the electrical gradient that most closely simulates the in vivo condition (6, 25).

The contribution of submucosal glands in vivo to resting volume flow across the trachea can be estimated from published data. Unfortunately, single-gland flows have been reported only in cats (28, 37). The resting flow of 9 $nl \cdot gland^{-1} \cdot min^{-1}$ multiplied by an average density of secreting glands in resting canine trachea of 15 glands/cm^2 (12, 13) and the canine tracheal surface area (60 cm^2) projects a secretion of 0.5 ml/h, a rate that approximates the estimated flow induced by the osmotic gradient.

However, there is little doubt that cholinergic mediators can induce in vitro (22) or raise in vivo (13) NaCl and water secretion by the tracheal epithelium. The increase in collected volume after methacholine (Table 6), the net NaCl secretion by in vitro trachea under open-circuit conditions (22), and the increase in "hillock" formation on the tracheal surface after electrical simulation of the superior laryngeal nerve (13) document this conclusion. Because the trachea is innervated in vivo but not in vitro, and the effects of cholinergic agents on electric PD in vivo (6) and in vitro (22) are minimal, we cannot dismiss a contribution of cholinergic tone to tracheal surface fluid volume and composition at rest. Reasor et al. (29), however, have reported that atropine did not alter the collection rate from screens in dog trachea, suggesting little tonic cholinergic influence on resting secretion.

The composition and volume of tracheal surface liquid may be further modified by the cephalad movement of bronchial surface fluid. In particular, the K^+ and HCO_3^- concentrations of tracheal surface fluid, which are in excess of that predicted by passive forces, support this widely held view (38). The isosmotic NaCl and volume additions to tracheal fluid after methacholine administration may also be due in part to the increase of flow from the bronchial surfaces.

The epithelium of subsegmental bronchi (~0.5 cm) appears to exhibit different transport and permeability characteristics. In addition to a lower transepithelial PD (6), NaCl concentrations of surface liquid are lower and HCO_3^- and K^+ concentrations are higher than those of the trachea. Our in vitro studies indicate that NaCl and, presumably, water are absorbed from bronchial surfaces under open-circuit conditions (9). Further, the genesis of the large transbronchial K^+ gradient may be explained by the active K^+ secretion that was detected in these studies (8). Our results suggest that, whereas water movement into the trachea may be driven largely by passive forces, solution movement in the bronchi is more complex. A portion of the surface liquid is probably transported by "mucociliary" clearance to central airways. Some airway liquid may be reabsorbed by active NaCl transport and in response to transepithelial colloid and hydrostatic pressure gradients. Finally, in certain circumstances such as exercise, bronchial surfaces may be recruited to serve as evaporative surfaces.

Despite several limitations, including the necessity to select sample volumes greater than 15 μl for some analyses and irritation of the mucosa, the filter-paper technique we describe offers several advantages over other methods. It is rapid and simple. It permits liquid from different regions of the airway surfaces of the same animal to be sampled, making it useful to assess changes in regional airway hydration and surface liquid composition in response to various stimuli, including physical trauma and pharmacologic and toxic agents. Finally, it should be possible to evaluate the concentrations of constituents of aerosols that are deposited on the airway surface.

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